Multiple *Cryptosporidium* genotypes detected in wild black rats (*Rattus rattus*) from northern Australia

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HIGHLIGHTS

► Molecular and microscopic detection of *Cryptosporidium* spp. in Australian wild rats.
► Detection of multiple *Cryptosporidium* sp. 18S rDNA genotypes.
► Detection of a novel *Cryptosporidium* sp. actin genotype.
► Phylogenetic characterization.
► Epidemiology.

GRAPHICAL ABSTRACT
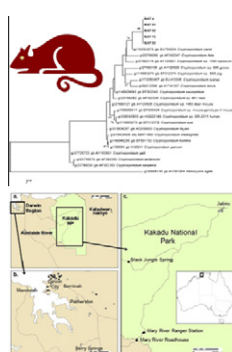
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ABSTRACT

As part of a broader investigation into the potential role of black rats (*Rattus rattus*) as disease vectors into native small mammal populations of northern Australia, blood and faecal samples from wild black rats were screened by molecular methods, for piroplasms (*Babesia* and *Theileria*), trypanosomes and the enteric parasite *Cryptosporidium*. While piroplasms and trypanosomes were not detected in the blood of these animals, the overall prevalence of *Cryptosporidium* 18S rDNA in faecal samples was 8.2% (7/ 85). Co-occurrence of multiple genotypes was observed in 57.1% of the infected individuals (4/7); cloning and re-sequencing resulted in 14 sequences which broadly grouped with *Cryptosporidium* sp. rat-genotypes II and III. A novel rat-derived *Cryptosporidium* sp. genotype at the actin locus was also obtained from five animals. The relatively low infection rate detected, and the epidemiological data on cryptosporidiosis, do not conclusively support a current threat to native Australian mammals from black rats carrying *Cryptosporidium*. However, this observation is based on sampling limited isolates, in limited regions. Further studies, also including sampling of native mammals, are required on larger sample sizes and from wider geographic areas, to determine the significance of these findings, including the public health importance of *Cryptosporidium* spp. from rodents.

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1. Introduction

The recent broad scale decline of small mammals across northern Australia has been cause for increasing concern, with data from Kakadu National Park surveys providing clear evidence of decline in both diversity and abundance (Woinarski et al., 2011). Whilst total grazing pressure, fire regimes, and feral predators likely contribute to these declines, observations from NRETAS and Kakadu NP (unpubl.) confirm the evidence that the black rat (*Rattus rattus*) has expanded into areas of native bushland during the same period as the small mammal declines (Griffiths, 1997; Woinarski, 2000).

Rats, as highly opportunistic, prolific and mobile animals, have capitalized on human movements to conquer most continents, and a variety of habitats (Watts, 2002). In many countries, they are recognized pests for multiple reasons including crop destruction, ecological disturbance through direct and indirect competition, predation and disease threats (Myers et al., 2000; Stokes et al., 2009a,b). The black rat, which originated in tropical mainland Asia and later spread to Europe and the rest of the world (Musser and Carleton, 1993), is now found throughout much of coastal Australia, including urban and peri-urban habitats. Although competitive interference by native rodent populations may have so far limited colonization of some areas (Stokes et al., 2009a,b), elsewhere black rats can represent a threat to native Australian rodents (Stokes et al., 2009a,b).

Further threats to native species come from the rats' capacity to act as vectors for novel or existing disease agents. A notable example includes the correlation between the introduction of black rats carrying the protozoan blood parasite *Trypanosoma*, and the extinction of the Christmas Island rat (*Rattus macleari*) in the early 20th century (Wyatt et al., 2008). Other pathogenic protozoan parasites, such as those in the genera *Theileria*, *Babesia* and *Cryptosporidium*, are also known to occur in Australia. *Theileria* and *Babesia* (a.k.a. piroplasms) are two ubiquitous intra-erythrocytic and morphologically-similar protozoa (order Piroplasmida, phylum Apicomplexa), causing piroplasmosis in several hosts, including humans (Criado et al., 2006; Homer et al., 2000). Piroplasms are mainly transmitted by tick vectors, and can cause significant economic losses for their high pathogenicity in domesticated animals like cattle, sheep, horses, and goats, and significant clinical disease in companion animals (Jefferies et al., 2007). Human babesiosis in the United States has been attributed to the rodent babesia *Babesia microti*, while in Asia piroplasms have been identified in bandicoot rats (*Bandicota indica*), spiny rats (*Rattus coxinga*), and wild mice (*Apodemus speciosus* and *A. argenteus*) (rev. in (Dantrakool et al., 2004)). *Cryptosporidium* spp., the most common enteric protozoan parasites infecting domestic animals and humans (Fayer et al., 2000; Xiao, 2010), have been found to infect common rat species, including the Norway rat (*Rattus norvegicus*) (Bomfim and Lopes, 1998; El-Wahed et al., 1999; Iseki, 1986; Kimura et al., 2007; Miyaji et al., 1989; Quy et al., 1999; Webster, 1996; Yamaura et al., 1990), the Tanezumi Rat (*Rattus tanezumi*) (Lv et al., 2009), and the black rat (*Rattus rattus*) (Chilvers et al., 1998; Miyaji et al., 1989; Rhee et al., 1991; Torres et al., 2000; Yamaura et al., 1990). Infections can spread efficiently by contamination of water sources with oocysts (Quy et al., 1999).

To investigate the potential role of black rats as disease vectors for these parasites into native small mammal populations of northern Australia, a survey of black rats was conducted in Kakadu National Park (12°40′25.13″S, 132°49′3.14″E), and across urban and peri-urban areas of Darwin, in the Northern Territory. Blood and faecal samples were screened by molecular methods, for haemoprotozoan species (*Babesia*, *Theileria* and trypanosomes) and the enteric parasite *Cryptosporidium*.

2. Material and methods

2.1. Trapping

Between March and August 2010, ten trapping sessions were conducted across a range of both urban and rural locations of Darwin and Kakadu National Park (Fig. 1). A total of 97 wild black rats were trapped. Table 1 provides information on the trapping effort in each session and in each trapping area, as well as the number of rats captured and time of capture.

Animals were trapped live in small cage traps (400 mm × 140 mm × 140 mm; Tomahawk Live Traps). In the first trapping session, aluminium box traps (325 mm × 100 mm × 100 mm; Elliott Scientific) were also used but found to be ineffective for black rats. A standard small-mammal bait mixture of peanut butter, honey and oats formed a staple bait base to which various ingredients were added, including sesame oil and fresh fruit pulp and rinds. Traps were set in the late afternoon and cleared each morning; they were closed during the day and rebaited every afternoon.

Animals were transported to a central processing point either in a cage trap covered with a large cloth bag, or in a new calico bag. If more rats were captured than could be processed that day, they were kept overnight in individual cages or large nesting boxes, provided with water and food *ad libitum* and kept well ventilated and well shaded. Rats were never housed together in the same cage or box.

2.2. Sample collection

Rats trapped in Darwin were anaesthetized at the Biodiversity Unit of the NT Department of NRETAS, using "The Stinger" portable anaesthetic machine (Advanced Anaesthetic Specialists, Australia), and isoflurane in oxygen (Forthane, Abbott Australasia, Australia), and then euthanized with pentobarbitone (Lethabarb, Virbac, Australia). Rats trapped in more remote locations (i.e. outside of Darwin suburbs and rural regions) were anaesthetized and euthanized in the field, using a temporary laboratory set up. Liquid nitrogen and a portable fridge were used to keep samples at appropriate temperatures during remote work.

Once anaesthetized, up to 3 mL of blood was collected by cardiocentesis. An aliquot of whole blood (0.5 mL) was placed in potassium EDTA-treated microtubes (Sarstedt, Germany) and stored at −80 °C for molecular analyses. Rats were euthanized with an intraperitoneal injection of 0.5 mL pentobarbitone (162.5 mg) diluted to 1.5 mL with water to prevent tissue irritation and limit *post mortem* artefact from euthanasia solution. A full physical exam was conducted: age, sex, weight and morphometric measurements were recorded. Body condition assessment was based on fat stores and muscling, and confirmed at *post mortem* examination. Faeces were collected at *post mortem* from the rectum into 1.5 mL tubes with sterile instruments, and stored at 4 °C for DNA analysis.

2.3. Molecular analyses

Molecular investigations were conducted at Murdoch University. Biological samples included: 86 blood samples and 85 corresponding faecal samples. Total genomic DNA was isolated, according to the manufacturer's instructions, from whole blood/EDTA (100 μL) and faecal pellets, using the MasterPure Purification Kit (Epicentre Biotechnologies, USA), and PowerSoil DNA Isolation Kit (MO BIO, USA), respectively.

Molecular assays, based on nested PCR protocols were used to detect the presence of specific DNA fragments of the small subunit
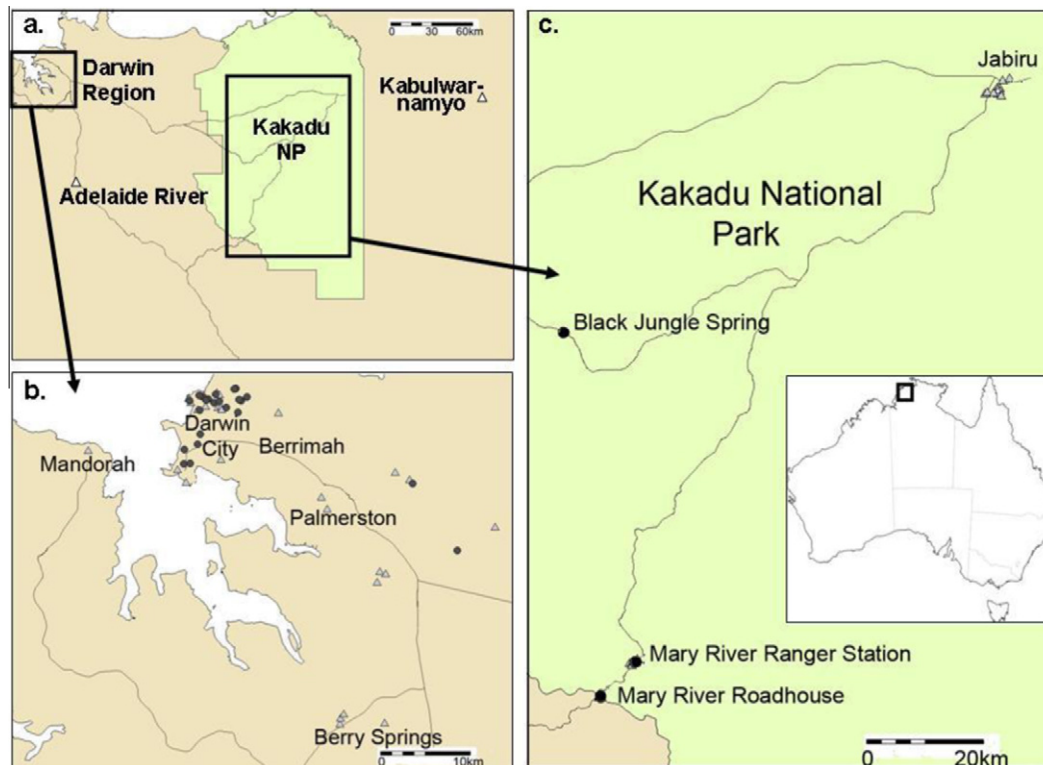
Fig. 1. Locations of the 2010 survey of black rats in the Northern Territory. Darwin–Kakadu region (a); Darwin region (b); Kakadu National Park (c). Inset shows the region within Australia generally. Triangles indicate trapping sites, filled circles are locations of black rats trapped and sampled.

Table 1
Trapping campaign.

Location	Trapping session										Total
	25–28 March	5–6 April	9–10 May	19–20 May	3–7 June	13–15 July	29–30 July	3–11 August	17–19 August	24–25 August	
Mary River Ranger Station	795 (1)							98 (1)			893 (2)
Mary River Roadhouse	186 (2)							122 (11)			308 (13)
Jabiru				490 (0)							490 (0)
Black Jungle Spring								200 (8)			200 (8)
Kabulwarnamyo								200 (0)			200 (0)
Adelaide River						140 (5)					140 (5)
Territory Wildlife Park						72 (0)					72 (0)
Darwin suburbs		81 (8)	116 (10)	63 (11)			75 (13)		90 (21)	45 (4)	470 (67)
Rural Darwin[*]				65 (0)			25 (1)		50 (0)	65 (1)	205 (2)
Total	981 (3)	81 (8)	116 (10)	128 (11)	490 (0)	212 (5)	100 (14)	620 (20)	140 (21)	110 (5)	2978 (97)

Number of trapping nights and captures of black rats (in brackets), at different locations around Darwin, and Kakadu National Park, in Northern Territory (year 2010).
[*] Rural Darwin includes the city of Palmerston, and outer suburbs such as Humpty Doo/Howard Springs, plus bushland areas such as Holmes Jungle, Howard Springs Reserve and Charles Darwin National Park.

ribosomal RNA (18S rRNA) gene of *Babesia* spp., *Theileria* spp., trypanosomes, and *Cryptosporidium* spp.

Piroplasms and trypanosomatid-specific 18S rDNA was screened for using PCR primers and conditions described previously (McInnes et al., 2011; Paparini et al., 2011, 2012). *Cryptosporidium* 18S rDNA was screened for using previously described primers (Xiao et al., 1999). When 18S rDNA positivity was detected, samples were also screened at the actin locus, using a hemi–nested PCR as previously described (Ng et al., 2006). All amplifications performed included negative and positive controls.

PCR products were run on a 1% agarose gel containing SYBR Safe Gel Stain (Invitrogen, USA), and visualized with a dark reader trans-illuminator (Clare Chemical Research, USA). PCR products corresponding to the expected length were excised, purified using

a MO BIO UltraClean DNA purification kit (MO BIO Laboratories, USA), and sequenced using an ABI Prism Terminator Cycle Sequencing kit (Applied Biosystems, USA), on an Applied Biosystem 3730 DNA Analyzer.

2.4. Cloning

Gel-purified PCR products providing mixed or low-quality DNA sequencing chromatograms were cloned in the pGEM-T Easy Vector System II (Promega, USA). After transformation of JM109 competent cells, plasmid DNA was extracted using the QIAprep Spin Miniprep Kit (Qiagen, Germany), from a subset of random clones, and sequenced as described above, using the SP6 and T7 promoter primers (Promega, USA).

Table 2
Cryptosporidium detection rate in wild black rats in northern Australia.

Location	Faecal samples (No.)	18S (No. positive)	Actin[*] (No. positives)	Detection rate (%)	
				18S (95% CI; SE)	[*]Actin (95% CI; SE)
Kakadu	20	0	0	0	0
Adelaide River	5	0	0	0	0
Darwin Region	60	7	5	11.7 (3.5–19.8; 0.041)	8.3 (1.3–15.3; 0.036)
Total	85	7	5	8.2 (2.4–14.1; 0.030)	5.9 (0.9–10.9; 0.026)

Detection rates of *Cryptosporidium* spp. in PCR products at the 18S rDNA and actin loci, in wild rat (*Rattus rattus*) hosts captured in the Northern Territory, Australia.
[*] Molecular analyses of the actin gene were performed only on samples that were positive at the 18S rDNA locus.

2.5. Phylogenetic analysis

Phylogenetic analyses were conducted on the sequences obtained during the present study and from GenBank. Sequencing chromatogram files were analysed by FinchTV 1.4 (http://www.geospiza.com/Products/finchtv.shtml), and imported into Bioedit Sequence Alignment Editor (Hall, 1999), and MEGA 5 (Tamura et al., 2011) for manipulations and alignments by CLUSTAL W (Larkin et al., 2007). Models with low BIC scores (Bayesian Information Criterion), identified by MEGA 5 (Tamura et al., 2011), were chosen to construct neighbour-joining (NJ) trees, using BIONJ (Gascuel, 1997) on the Phylogeny.fr platform (Dereeper et al., 2008). Estimates of evolutionary divergence between sequences (*p*-distance) were calculated using MEGA 5 (Tamura et al., 2011).

3. Results

3.1. Protozoan DNA detection rate

All blood samples screened by PCR were negative for *Babesia* ssp., *Theileria* ssp., and trypanosomatid 18S rDNA. The prevalence of *Cryptosporidium* spp. 18S rDNA was 8.2%, with 7/85 faecal samples (from an equal number of animals) positive by PCR (Table 2). All the 7 positive animals (Rats 15, 25, 73, 81, 83, 93, and 94) were captured in the Darwin Region. Among them, 4 appeared to have mixed infections on the basis of multiple peaks in the sequencing chromatograms (Rats 25, 81, 83, and 93), indicating that multiple parasites' genotypes may be harboured within the same host. At the actin locus, *Cryptosporidium* was detected by PCR in 5/85 faecal samples (5.9%), from an equal number of animals, all of which were also positive at the 18S locus (Rats 15, 73, 81, 83, and 93).

3.2. Sequence analysis and phylogeny

Cryptosporidium DNA, amplified from isolates 25, 81, 83 and 93 was cloned, resulting in a total of 14 partial 18S rDNA sequences, with lengths ranging from 780 to 1193 bp. An NJ phylogenetic tree at this locus was reconstructed using the K2P substitution model (Kimura, 1980) (Fig. 2).

Cryptosporidium 18S sequences obtained from the wild black rats, broadly grouped with rat-genotypes II and III (Lv et al., 2009). Four sequences (Rat 81 clones 1 and 2, Rat 83 clone 4, Rat 93 clone 1) were rat-genotype III (99.8–100% identical). Three sequences (Rat 15, Rat 25 clone 4, and Rat 93 clone 3) were identical to each other and exhibited 0.4% genetic difference from rat-genotype III.

The remaining 7 sequences (Rat 25 direct sequence, Rat 25 clone 2, Rat 73 direct sequence, Rat 81 clone 4, Rat 83 clone 1, Rat 93 clone 4, and Rat 94 direct sequence) clustered with rat-genotype II, but exhibited 0.6–0.7% genetic distance from this genotype.

At the actin locus five un-cloned sequences were obtained from Rats 15, 73, 81, 83 and 93, which ranged in length from 757 to 797 bp and in the final de-gapped alignment (26 nucleotide sequences; 492 positions) they were 100% identical. No mixed sequences were detected. Unfortunately no actin sequences for rat-genotypes I–IV were available on GenBank for comparison, but the rat-derived isolates formed a novel strongly supported clade (bootstrap value 99%), with 10.3% genetic distance (51 SNPs/535 positions) from the *C. canis* coyote-genotype (Xiao et al., 2002) (Fig. 3). As mixed infections were found in several of the rats, it is not possible to identify this actin sequence as rat-genotype II or III. To the best of our knowledge this is the first rat-derived *Cryptosporidium* sp. genotype for the actin locus.

3.3. Geographic distribution and clinical results

All rats positive for *Cryptosporidium* at the 18S locus were captured in the Darwin Region and came from only three locations, from a total of 21 locations trapped within the Darwin suburbs (Fig. 4). Two were recorded from separate locations in May 2010 and five infected rats were captured in August 2010; one from one of the locations from May and four from a new location not previously trapped. All rats positive for *Cryptosporidium* were males (7/7), a sex ratio that is significantly different to that of the population sample (59 males: 38 females; *p* = 0.046, two-tailed Fisher's exact test). Age classes and body condition scores were unremarkable when compared to the distribution in the broader population, with age classes ranging from sub-adult (2/7) to adult (5/7), and body condition scores including average (1/7), good (5/7), and very good (1/7).

Endoparasites were found in six of the seven rats positive for *Cryptosporidium*. These included *Gongylonema cf neoplasticum* (5/7), *Eucoleus* spp. (2/7), an acanthocephalan (1/7), and unidentified helminth parasites of the small intestine (1/7), and large intestine (1/7). These endoparasites were only isolated from rats captured in the Darwin Region but rats carrying endoparasites were not significantly more likely to be positive for *Cryptosporidium* than endoparasite-free rats in the Darwin Region.

4. Discussion

In the present study, while piroplasms and trypanosomes were not detected in the blood of feral black rats from northern Australia, the overall prevalence of *Cryptosporidium* spp. in these animals was 8.2%, or 11.7% if only rats from the Darwin region are considered. This is within the reported range of 2.1–63% for *Cryptosporidium* prevalence in rodents (Feng, 2010; Feng et al., 2007; Foo et al., 2007; Lv et al., 2009; Morgan et al., 1999). Previous studies have highlighted how the prevalence of *Cryptosporidium* in common, brown, and black rats, can vary greatly. The lowest prevalence was 2.1% in brown rats from Japan (*n* = 48) (Yamaura et al., 1990), and the highest was 63.0% in wild black rats (*n* = 73), trapped on nine rural farms around Oxfordshire, United Kingdom (Webster and Macdonald, 1995).
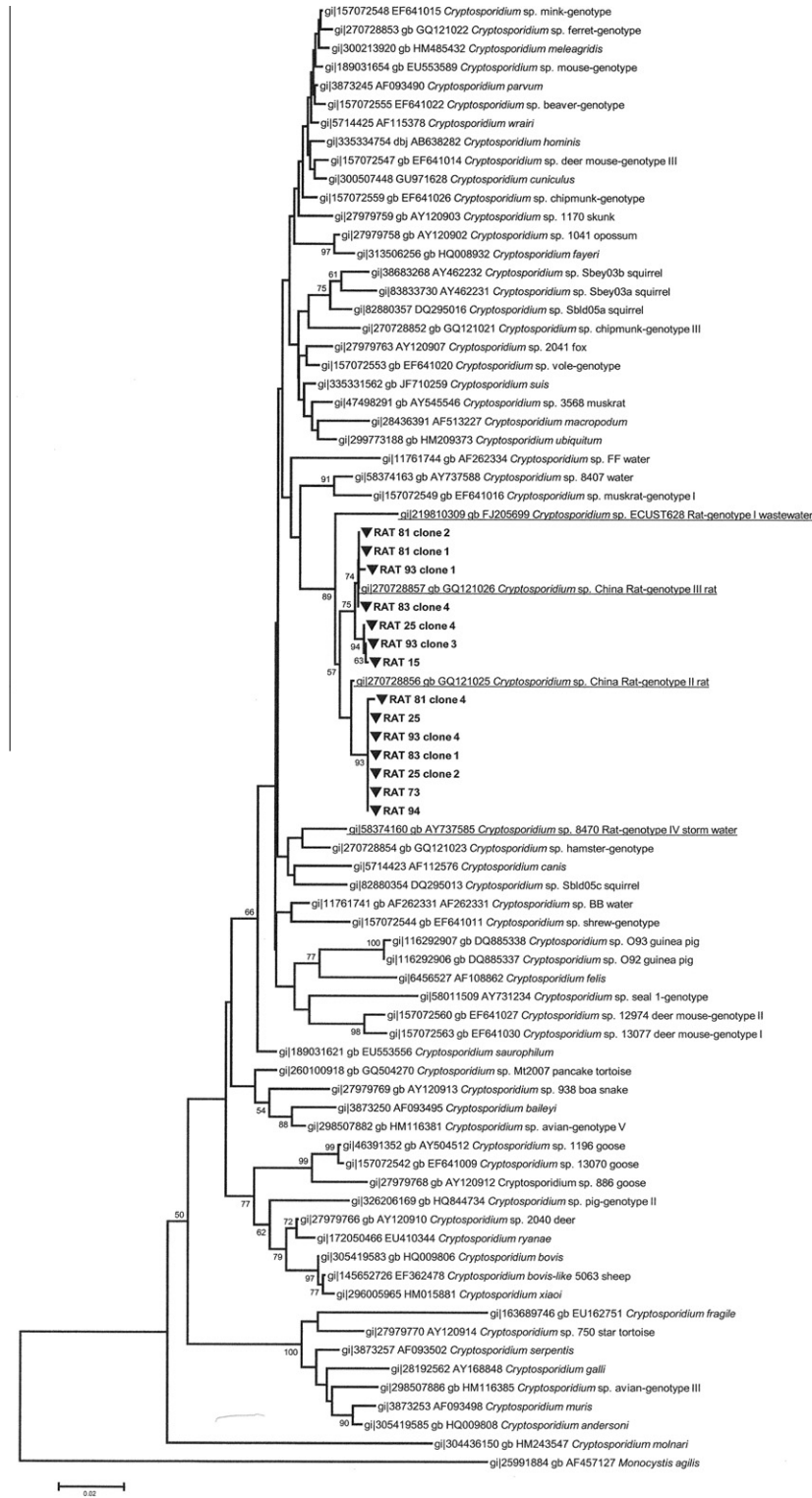
Fig. 2. Phylogenetic analysis of *Cryptosporidium* spp. based on 18S rDNA partial sequences. Evolutionary history was inferred using the neighbour joining method. The evolutionary history was inferred using the neighbour-joining method. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees, in which the associated taxa clustered together in the bootstrap test (1000 replicates), is shown next to the branches.

Rats that tested positive for *Cryptosporidium* were recorded only within the Darwin suburbs, and only at three of the 21 locations trapped in this area. This investigation was essentially an exploratory one, not designed to track prevalence or persistence of disease agents at specific locations, nor the epidemiology of *Cryptosporidium* in rats of this region. However, at one location

Cryptosporidium was detected in rats caught three months apart. This spatial–temporal pattern suggests that some individuals act as reservoir hosts (continually or periodically shedding microorganisms), a contaminated environmental source (e.g. water) sustains infections, or other species play a role in the transmission cycle, thus enabling persistence.
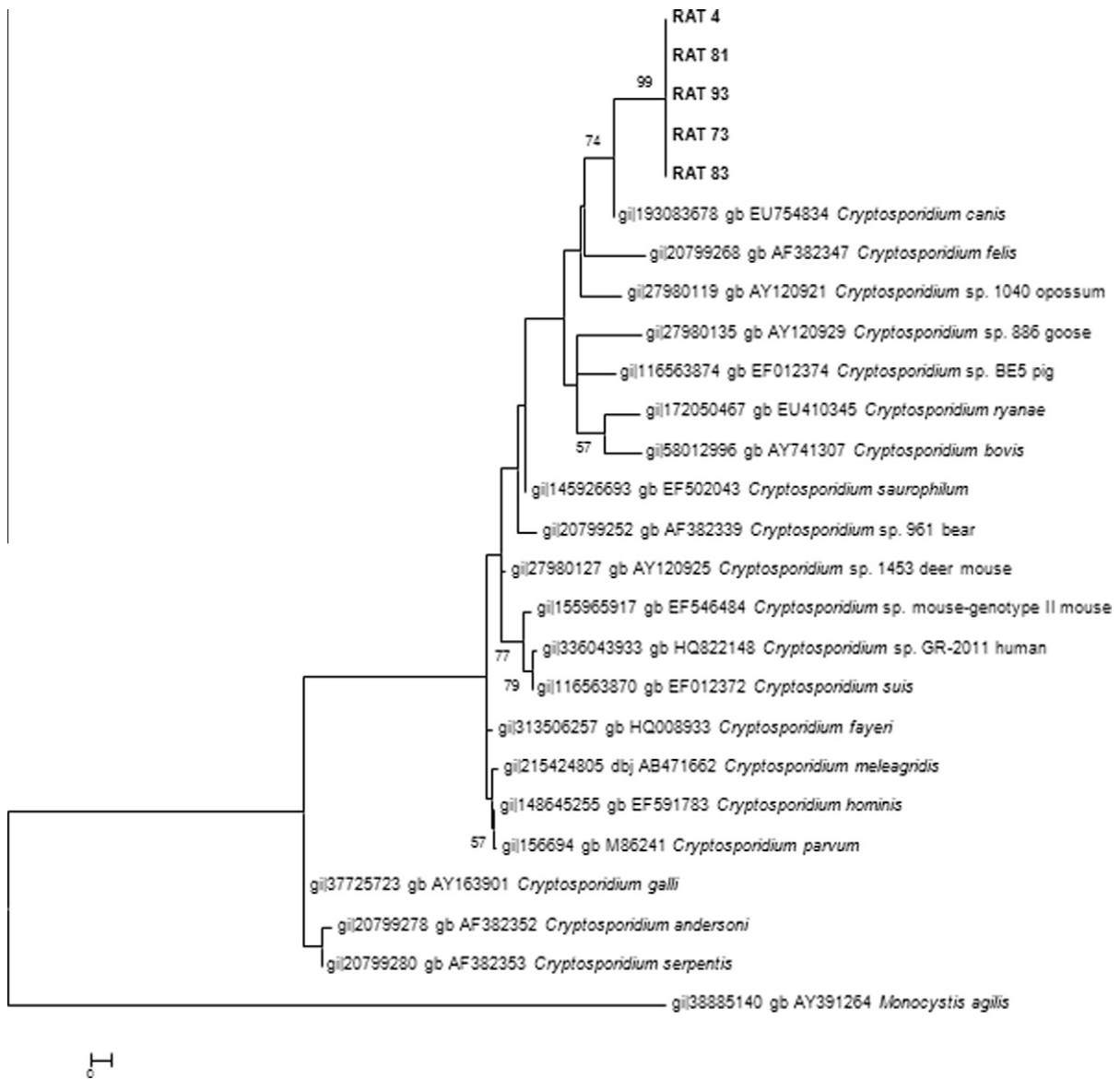
Fig. 3. Phylogenetic analysis of *Cryptosporidium* spp. based on actin partial sequences. Evolutionary history was inferred using the neighbour joining method. The evolutionary history was inferred using the neighbour-joining method. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees, in which the associated taxa clustered together in the bootstrap test (1000 replicates), is shown next to the branches.

In immuno-competent humans infections are usually cleared within 10–14 days with subsequent immunity to re-infection (Thompson et al., 2005); however, immuno-compromised humans, as well as domestic pets including dogs and cats, have been reported to remain infected for months or longer (Irwin, 2002; Olson et al., 2003).

It is interesting to note that no rats (0/20) tested positive for *Cryptosporidium* outside of the Darwin Region. This may be an artefact due to the small sample size, but, interestingly, it also coincides with the absence of endoparasites in the animals from the Kakadu National Park from the broader study. The lack of general parasitism of black rats in more remote or bush areas may relate to epidemiological factors, such as population density, number of susceptible individuals and opportunities for interactions, and/or environmental effects on parasite survival and transmission outside of the host. It may also reflect the parasite burdens of founder individuals for these remote populations. The Enemy Release Hypothesis describes the process of parasite loss during invasion or expansion of host ranges, and is theorised to infer a competitive

advantage to invading species due to lower general parasitism (Prenter et al., 2004).

Prior to genotyping studies, it was thought that rodents were infected with *C. muris* and *C. parvum* (Feng, 2010). However, it is now believed that most infections in house mice are caused by *C. tyzzeri* (formerly mouse-genotype I), which differs significantly from *C. parvum* (Ren et al., 2011, Xiao et al., 2004). Thus, house mice are commonly infected with *C. muris* and *C. tyzzeri*, and occasionally with the mouse-genotype II (Foo et al., 2007; Morgan et al., 1999; Morgan et al., 2000). Confirmed *C. parvum* infections have been reported in only a few rodents (Feng, 2010; Lv et al., 2009; Morgan et al., 1999).

Several species/genotypes have been identified in rats, including *C. tyzzeri* plus four rat-genotypes (I–IV) (Lv et al., 2009). Rat-genotype I has previously been identified in a *Boa constrictor* in the US (Xiao et al., 2004), and in water samples from Shanghai (Feng et al., 2009), and the UK (Chalmers et al., 2010). Rat-genotypes II and III have previously been described from brown rats (*R. norvegicus*) and Asian house rats (*R. tanezumi*) from China (Lv
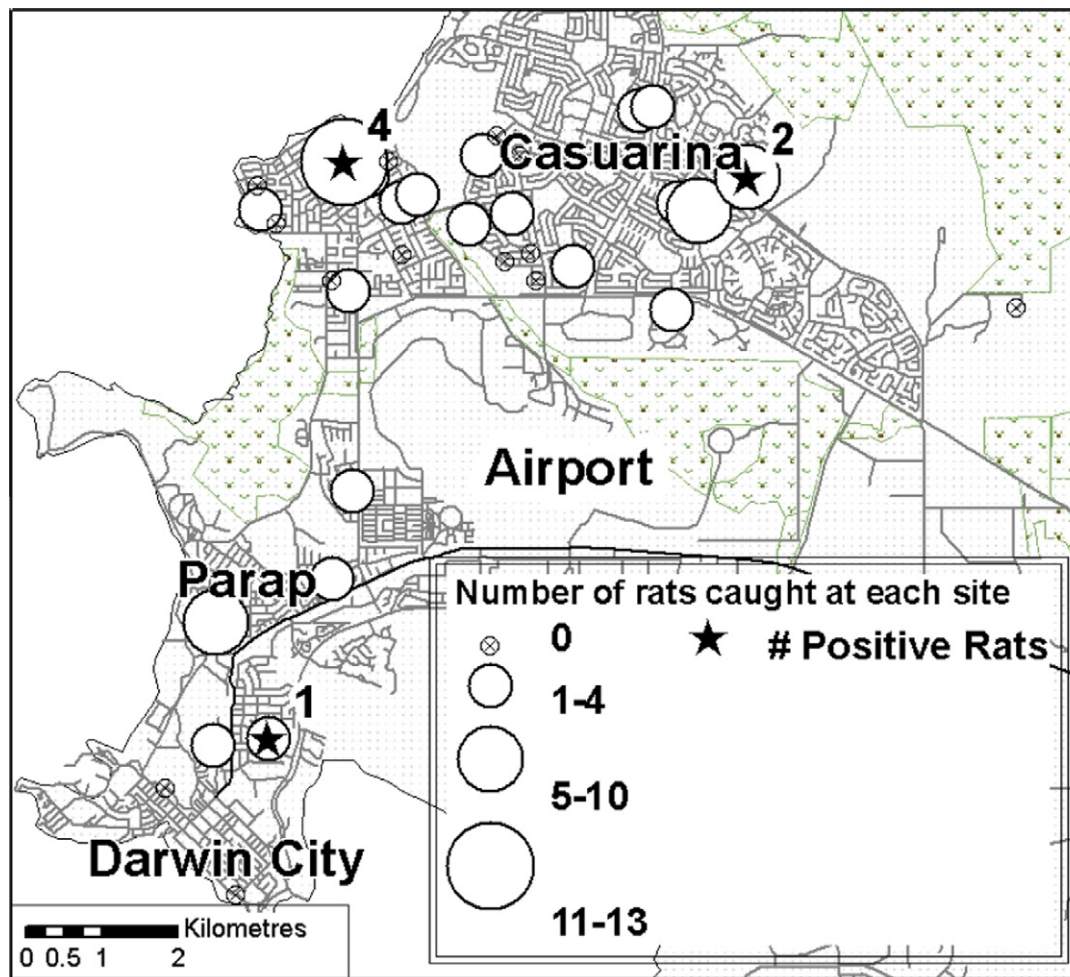
Fig. 4. Geographic distribution of trapping locations in the Darwin suburbs, in 2010. The number of rats trapped at each location is proportional to the radius of each bubble. Sites where *Cryptosporidium* spp.-positive animals were found are indicated by a star, and the number of infected animals is also indicated.

et al., 2009). Rat-genotype IV (previously W19) has been identified in storm-water (Jiang et al., 2005; Lv et al., 2009). This is the first report of 18S *Cryptosporidium* spp. rat-like genotypes from Australia.

In the present study, *Cryptosporidium* 18S sequences obtained from the wild black rats, broadly grouped with rat-genotypes II and III but exhibited significant variation, with 4 sequences identified as rat-genotype III (∼100% homology), and the remaining sequences forming 2 clades (bootstrap values ⩾78%), one grouping with rat-genotype III, and one with rat-genotype II. All 5 sequences obtained for the actin locus were identical, either suggesting that rat-genotype II and III are actually one genotype exhibiting variability at the 18S locus, or that multiple infections were present in the rats, but only one genotype was amplified at the actin locus (the first rat-derived *Cryptosporidium* sp. genotype for the actin locus). Further studies are required to determine this. Mixed infections were observed in 57.1% of the infected individuals (4/7). This fig. appears much higher than in previous studies, which reported mixed infections in 10.8% of rodents screened in China (Lv et al., 2009), and 4.3% in wild rodents in Spain (Torres et al., 2000).

The general health of rats did not appear to be adversely affected by infection with *Cryptosporidium,* although sample sizes were too small to carry out robust statistical analyses. In general, mortality may also remove infected rats from the population before they can be sampled, thereby biasing the results towards those animals that have adequate host immunity. Black rats in the Dar-

win region co-exist with a variety of domesticated animals, as well as humans, native mammals (rodents and marsupials) and other wildlife. Black rats are anecdotally present in large numbers in urban Darwin, as supported by the success of trapping in this region. The potential for zoonotic or spill-over events into other species is therefore high, and without surveying all sympatric species, it is not possible to conclude that the *Cryptosporidium* species found in this study are confined to black rats, nor to infer the pathogenicity of this strain for native mammals from northern Australia.

There have been limited molecular surveys on the prevalence and pathogenesis of *Cryptosporidium* spp. in Australian native mammals; for marsupials, however, results to date suggest that most species found are host-adapted, although *C. parvum* and *C. hominis* have been reported (Hill et al., 2008; Ng et al., 2011; Power, 2010).

Recent findings of clinical disease in a human from the presumed marsupial-specific *C. fayeri* (Waldron et al., 2010), and cattle-genotypes present in Western Grey Kangaroos (*Macropus fuliginosus*) (Yang et al., 2011), suggest that the zoonotic risk and multiple host potential of *Cryptosporidium* may be greater than previously thought. Further, native mammals in northern Australia are currently undergoing significant declines (Woinarski et al., 2011), with potential inbreeding depression due to isolated small populations and genetic bottlenecks. This may render them more susceptible to previously host-adapted or non-pathogenic disease entities.

The results of this study provide no evidence that rats play an important role as vectors for haemoprotozoa into native small mammal populations. Although *Cryptosporidium* may not be a direct cause of broad scale declines, this study highlights the limited data on these and other protozoans in both native and domesticated mammals of northern Australia. Results were based on sampling of a limited number of isolates, in limited regions, and did not include sampling of sympatric native mammals. Further research is required to assess the prevalence of haemoprotozoa and enteric parasites, in larger numbers of rats from wider geographic areas, in order to elucidate their potential impact on native mammals, the public health importance of *Cryptosporidium* spp. from rodents, and the role of these animals in the transmission of human cryptosporidiosis. Ideally these studies should include native mammals from a broader taxonomic range, to further understand the epidemiology of protozoan parasites in northern Australia. However, such studies will be restricted by the decline of many native mammal species across the north.

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